510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k040586

B. Analyte:
Anti-neutrophil cytoplasmic antibody (ANCA)

C. Type of Test:
Semi-quantitative, ELISA

D. Applicant:
RhiGene, Inc.

E. Proprietary and Established Names:
MESACUP Test MPO

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660 Multiple Antibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MOB (Anti-neutrophil cytoplasmic antibodies (ANCA)
4. Panel:
   Immunology

G. Intended Use:

1. Intended use(s):
   The MESACUP Test MPO is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-myeloperoxidase (MPO) antibodies in human serum.

   Clinical (hospital and reference) laboratory personnel are the intended users of the MESACUP Test MPO.
2. Indication(s) for use:
   The intended use of the MESACUP Test MPO is as an aid in the diagnosis of certain systemic vasculitides such as microscopic polyarteritis and crescentic glomerulonephritis.
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:
   None

H. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) using microtiter plates as the solid phase. The plate wells are coated with MPO antigens, which allow anti-MPO antibodies to react with the immobilized antigen (sample). The conjugate is polyclonal goat anti-human IgG (heavy chain specific) horseradish peroxidase (HRP), which uses 3,3'5,5' tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H_2O_2) as substrate. The kit contains two levels of calibrators (0 units/mL and 100 u/mL) for interpretation of results. A positive and a negative control are included

with the kit. The kit also contains sample diluent, wash buffer concentrate and stop solution.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   The Binding Site Bindazyme Human Anti-MPO Enzyme Immunoassay Kit
2. Predicate K number(s):
   k981030
3. Comparison with predicate:

Similarities		
Item	**MESACUP Test MPO**	**Predicate**
Indications for Use	For detection of IgG anti-MPO antibodies as an aid in the diagnosis of certain systemic vasculitides such as microscopic polyarteritis and crescentic glomerulonephritis.	
Assay principle	Indirect ELISA	
Analyte	IgG anti-MPO antibodies	
Sample matrix	Serum	
Substrate	One-component TMB	
Differences		
Item	**MESACUP Test MPO**	**Predicate**
Cut-off	22 U/mL	9 U/mL
Detection range	5 - 200 U/mL	1.23 - 100 U/mL
Assay time	150 minutes at Room Temp	90 minutes at Room Temp
Absorbance	450 nm / 620 nm	450 nm
Conjugate	HRP-goat anti-human IgG	HRP-rabbit anti-human IgG

J. Standard/Guidance Document Referenced (if applicable):

Not applicable

K. Test Principle:

The assay involves enzyme-linked immunosorbent assay (ELISA) technology. Calibrators and patient sera are incubated with MPO antigens for a specified time, and then washed. This step is followed by incubation with horseradish peroxidase conjugated anti-human IgG. The reaction is then washed, stopped, and the color is allowed to develop and measured photometrically.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
   Three lots of the MESACUP Test MPO were used to determine the intra-assay, inter-assay and inter-lot value precision for the test.

   Intra-assay
   Intra-assay precision (% CV) was determined by running 2 serum samples (i.e., low and high positive) using one dilution and 40 replicates on 3 separate assays (3 separate lots). Three separate plates were randomly selected from each plate-coating run (kit-lot). The mean % CV for intra-assay precision for the samples tested on 3 plates from each lot was 3.6% (Range: 3.0 – 4.3%).

Inter-assay, intra-lot

To determine the amount of variability between plates of the same lot, 2 samples in duplicate were tested on 7 separate assays from the same plate lot for each one of 3 separate plate lots. The mean % CV for inter-assay, intra-lot precision was 6.7% with a range of 4.7 – 7.8%.

Inter-assay, inter-lot

The precision between lots was determined by comparing the values recovered for 2 different samples on 3 different pilot lots. Each of the 3 samples was tested in 40 replicates on one plate from each lot. The mean inter-assay, inter-lot % CV was 3.6%.

b. Linearity/assay reportable range:

The reportable range of 5-200 U/mL was demonstrated by recovery studies. Linearity is not claimed for this assay.

c. Traceability (controls, calibrators, or method):

An international reference material for anti-MPO antibodies is not available. The assay is calibrated in relative arbitrary units (U/mL).

d. Detection limit:

Not applicable.

e. Analytical specificity:

Several substances were added to each of three patient specimens (i.e., negative, moderate, and high) to test for interference. Based on the results summarized below, the addition of these substances at the levels tested did not affect the assay results.

Substance	Level Range (U/mL)	Low			Moderate			High		
		Mean (U/mL)	***SD***	***%CV***	***Mean (U/mL)***	***SD***	***%CV***	***Mean (U/mL)***	***SD***	***%CV***
Hemoglobin	0 – 440	5.5	0.3	5.9	35.9	1.9	5.2	76.2	6.4	8.4
Bilirubin C	0 – 19.5	6.1	0.2	3.1	38.5	2.3	5.9	82.3	5.0	6.1
Bilirubin F	0 – 18.6	5.9	0.2	3.8	38.1	2.5	6.6	85.3	7.5	8.8
Chyle	0 – 2350	5.9	0.5	8.4	34.0	2.0	5.9	90.1	6.7	7.5
Rheumatoid Factor	0 - 500	6.2	0.5	7.9	37.8	2.5	6.5	89.4	6.0	6.7

f. Assay cut-off:

Sera from patients with systemic vasculitis (n= 79) were tested by indirect immunofluorescence (IIF) for the presence of anti-neutrophil cytoplasmic antibodies (ANCA). Positive samples were classified as either cytoplasmic or perinuclear ANCA. The results of testing with the MESACUP Test MPO were compared to those from p-ANCA IIF positives since "*the major binding agent for positive p-ANCA antibody specimens"* in the IIF method is myeloperoxidase. The best performance was observed at an assay cut-off of 22 U/mL. There is no equivocal (gray) zone for this assay.

g. Stability:

Three manufacturing lots of the device were used in real time stability studies. The kits were stored at 2 – 8 °C. Six samples were tested at 3, 4, 5, 6, 9, and 11 months, and evaluated for optical density (O.D.) value recovery per the established assay specifications. The kits were stable up to 11 months under the specified experimental conditions.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   The tables below shows the results of comparison of serum samples (N=159) that were tested with the MESACUP Test MPO and the predicate device.

	Bindazyme +	**Bindazyme -**
MESACUP-MPO +	**26**	**3**
MESACUP-MPO -	**4**	**126**

STATISTIC	**Value**	**95% CI**
Prevalence	0.1887	0.1279-0.2495
Positive Agreement	0.8667	0.7450-0.9883
Negative Agreement	0.9767	0.9507-1.0028
Total Agreement	0.9560	0.9241-0.9879

   b. *Matrix comparison:*
   Serum is the only recommended matrix.
3. Clinical studies:
   a. *Clinical sensitivity:*
   Clinical sensitivity for the MESACUP-2 Test MPO was determined by testing sera (n = 79) from patients suspected to have a systemic vasculitis disease. Using 22 U/mL as the cut-off, 36.7% (29/79) of the samples were positive for anti-MPO antibodies. The mean value for this sample population was 46.7 U/mL (SD = $\pm$ 75.7). Single Factor ANOVA analysis that compared this value to the mean for the healthy controls gives a p-value of 4.03×10^{-8}. Therefore, at a level of $p<0.05$ for statistical significance, the results of this population were determined to be statistically different compared to the healthy controls.
   b. *Clinical specificity:*
   The applicant evaluated samples, in duplicate, from 80 consecutive healthy blood donors and used these samples as the normal population. The mean value was 1.1 U/mL (SD = $\pm$ 0.9). None of the samples tested positive in this sample population. Therefore, the specificity was 100 %.

Similar studies were performed with samples from patients from various autoimmune diseases. The table below provides a summary of the results obtained in these subgroups.

Disease or Disease Status	N	Mean (U/mL)	SD	Specificity (%)
Rheumatoid Arthritis	9	0.32	0.24	0
Sjögren's Syndrome	10	9.58	6.56	0
Systemic Lupus Erythematosus	10	7.54	9.21	10.0
Mixed Connective Tissue Disease	10	5.37	3.44	0
Polymyositis/ Dermatomyositis	10	3.19	3.94	0

The absence of significant cross-reactivity demonstrates good specificity of the test for the intended indication

c. Other clinical supportive data (when a and b are not applicable):
Not applicable.

4. Clinical cut-off:
   See assay cut-off.
5. Expected values/Reference range:
   The expected value in the normal population is negative.

M. Conclusion:

Based on information in the submission, the MESACUP Test MPO is recommended as substantially equivalent to the predicate device regulated under 21 CFR §866.5660 Multiple Antibodies Immunological Test System (class II, product code – MOB, product name - antineutrophil cytoplasmic antibodies (ANCA) test system).